REMARKS

In the Office Action the Examiner repeated the objection to the Specification because of the presence of hyperlinks. Applicants are puzzled by this because the links were removed in the amendment of 24 March 2003. Perhaps this is an oversight on the part of the Office. However, the amendment removing the hyperlinks has been repeated above just in case the first amendment was not entered properly. Kindly disregard the amendment to the Specification if the hyperlinks have already been deleted.

The Examiner has reconsidered the restriction requirement and has withdrawn the restriction requirement between the mRNA and the Laminin α-4 subunit peptide. Where appropriate, claims drawn to the protein have been restored to their original form in this amendment.

Rejections under 35 U.S.C. 112, first paragraph.

In the Office Action the Examiner repeated the rejection of Claims 1-10, 13-18, 21-29, 32-36, 44-45, 48-68 and 75-78 under 35 U.S.C. 112, first paragraph as containing subject matter not described in the specification in such a way as to enable on skilled in the art to make and/or use the invention. The Examiner largely rejected Applicants traverse made in the amendment of 24 March but did conclude that the claims drawn to gliomas are enabled. The Examiner continued the rejection of the claims directed to methods for predicting recurrence of tumors and for establishing grades of tumors. Those points will be discussed below; however, it is important to note that while evaluating the Examiner's statements, the undersigned Attorney began to wonder if the Examiner had received a legible version of the drawings. Apart

from intensity information reported in tables in the specification, much of the comparative data is present in the figures in the form of bar graphs. The original informal drawings are very difficult to make out. The undersigned attorney recently obtained this case from another law firm. In the file was a set of formal drawings with a note indicating they were for the Examiner. However, it was not possible to determine whether the drawings had ever been submitted. A telephone conversation with the Examiner established that the formal drawings did not appear to be in the case. Therefore, copies of the formal drawings are submitted herewith for the Examiner's use. Applicants believe that the improved clarity of these drawings should make it much easier for the Examiner to verify the points made below.

Setting aside for the moment the enablement rejections raised against many of the claims, Applicants are somewhat puzzled by the continued rejection of Claim 18 and the claims depending therefrom. That claim is directed strictly to gliomas so it would seem to be allowable according to the Examiner's statement in the second paragraph of page 6. If the Applicants' understanding of the Examiner's statement that claims to gliomas are enabled is mistaken, they would appreciate being corrected.

As in the immediately previous amendment, Applicants respectfully traverse these rejections and will address the points made by the Examiner. The inventive method is based on the discovery that of the plethora of genes up-regulated and down-regulated in a variety of malignancies (note that the Declaration was presented to indicate that the results had been extended beyond gliomas at the time of filing and have since been confirmed), one up-regulated gene common to a range of malignant tissues (and hitherto not

known to be associated with malignancy) is the gene for the α4 subunit of laminin. Further, Applicants also demonstrated that malignant and pre-malignant tissues actually express the laminin α4 peptide. The introduction to the application explains that this particular peptide is associated with certain embryonic tissues and is found at relatively low levels in a number of normal tissues. The highest levels in normal tissues are found in intestine, skeletal muscle, liver, lung and ovary. However, only weak expression has been observed in pancreas, testis, prostate, spleen, kidney and brain (page 6 of specification).

Although the Examiner renewed most of the rejections, Applicants are gratified by the Examiner's finding the detection of malignancy for gliomas is enabled. However, the Examiner difficulties with the Declaration of Dr. Ljubimova should be explored. Applicants apologize for presenting data that was not directed to the restricted claims. This was unintentional because the Applicants view the invention as a whole; this was also unavoidable because most of the recently gathered data are based on the protein. Once the gene arrays had been used to identify the up-regulation of the laminin α4 gene, and once it was demonstrated that the laminin α4 peptide was synthesized in increased amounts, it became much simpler to look for the protein rather than the nucleic acid. Fortunately, this gaffe is mooted by the Examiner's reconsideration of the restriction between protein and nucleic acid. In discounting the Declaration the Examiner seemingly becomes confounded by the presence of either Laminin 8 or Laminin 9. It is true that both of these proteins share the same alpha subunt (α4) whereas they differ in the beta subunit. Applicants have difficulty in understanding the Examiner's statements concerning Laminin 9 not being correlative and "that the alpha4 may not be

responsible for the expression in breast tissue." (page 7 of the Office Action). By "the expression" Applicants understand the Examiner to be inquiring whether the strong expression of α4 actually controls the expression of Laminin 8 (as opposed to control by the β1 subunit). The analyses presented in the application demonstrate that α4 transcription is up-regulated resulting in increased translation of the α4 peptide and the appearance of Laminin 8. The cells in which Laminin 8 appears also produce the other subunits necessary for assembling Laminin 8, but the key seems to the α4 subunit. While not conclusively established by these studies, synthesis of the other subunits may be in response to the α4 synthesis. What is fairly clear, however, is that certain normal tissues (and certain abnormal tissues) produce certain subunits and not other subunits (see pages 49-52 of the application). There is no evidence that the β1 subunit drives or controls the expression of the α4 subunit.

The Examiner remarks that unlike the application, the Declaration has not provided any intensities. Table 1 in the Declaration is a summary of immunocytochemical staining results. Paragraph 9 of the Declaration describes the intensity of the staining reported in Table 1. Note that the Laminin 9 expression (which has so confounded the Examiner) in the two normal breast tissues is described as "weak" Sixteen of 19 invasive breast carcinomas and all 3 metastases showed strong expression of laminin-8 chains. Intensity information is also provided in the western blots accompanying paragraph 9. The normal breast sample show little if any α4. Also, very little β1 is present (the second normal breast has an artifact right above the β1 band whereas the first normal breast does show some β2 (which might explain the weak Laminin 9 staining).

In contrast the two metastasized breast cancers show strong expression of $\alpha 4$ and $\beta 1$. These patterns are consistent with the discoveries that form the basis of the present invention. That is, overexpression of $\alpha 4$ results in a cancer cell that is particularly invasive and particularly likely to metastasize. Thus, of the invasive breast cancers tested all 19 showed overexpression of $\alpha 4$ (this is the invasive/aggressive) indicator, and 16 showed Laminin 8 (this is the metastasis indicator—all metastasized cancers showed Laminin 8). The non-invasive cancers showed a preponderance of Laminin 9. Normal breast tissues show a low level of $\alpha 4$ which did not come close to the levels in malignant tissue. Applicants believe that the Declaration clearly shows that the invention is readily applicable to breast cancer as well as brain cancer. It does not take undue experimentation to apply the methods taught in the application to establish normal levels of $\alpha 4$ expression in breast tissue. Breast tissue showing significantly elevated expression of $\alpha 4$ are malignant with higher levels of $\alpha 4$ indicating increasing aggressiveness of the cancer. Very high levels of $\alpha 4$ and the presence of Laminin 8 are markers for an aggressive tumor that has a very high potential for metastasis. Prior to this discovery, it was as feasible to predict metastatic potential based on histology alone. Thus, Applicants respectfully request that the Examiner withdraw the rejections under 35 U.S.C. §112, first paragraph. Even if the Examiner continues to hold the invention to be not enable for "any" malignancy, it is certainly enabled for both brain malignancies and breast malignancies.

Applicants now wish to address the rejection of claims addressed to recurrence of malignancy. In the immediately previous amendment Applicants extensively discussed the methods by which normal levels for various genes were expressed in brain tissue. Applicants demonstrated that corpus callosum

could be used as a "normal" control since it was not ethically acceptable to take control samples of brain tissue from the patients. Rather, the primary tumor was excised along with a "buffer zone" of surrounding tissue. This is normal procedure for such cancers. The tumors were analyzed cytologically as well as by the inventive method. The surrounding non-tumor tissue was also analyzed cytologically and by the inventive method. The Examiner is directed to the discussion and data on pages 44-54 of the application. In these pages it is clearly reported that histologically normal adjacent tissue showed abnormal gene expression. This expression was sometimes similar to the primary tumor but not identical. The reason for this expression is as yet unknown. Perhaps there are tiny areas of tumor cells in the adjacent tissue; however, such cells have never been detected histologically. Perhaps the primary tumor's secretion of growth factors causes the adjacent cells to show abnormal gene expression. What is significant is that the pattern of abnormal gene expression predicts the recurrence of the tumors. For example, patient 16 showed lower levels of genes related to gliomas than did patient 39. When these patients were followed up; patient 16 had much slower recurrence of tumor than did patient 39. Significantly, these comparison data are presented in figures of which the Examiner hitherto had largely illegible copies. Applicants believe that now that the Examiner is able to verify these data, the Examiner will agree that the instant invention is enabled for a method of predicting recurrence. Furthermore, (see top of page 45) data are presented for an additional seven patients which data verify the same ability to predict recurrence. Therefore, Applicants respectfully request that the rejections of Claims 28, 44 and 53 be withdrawn.

The claims drawn to establishing a "grade" are also supported by pages 44-54 of the specification and the accompanying figures. The discussion of the data indicates that the more aggressive tumors (those that grew most rapidly) were also the ones that recurred the most rapidly. The significant point is that traditional ranking or grading of tumors is based strictly on histological features, but as discussed in the specification, histological features were unable to correctly identify aggressiveness and tendency to recur. The Examiner points out (page 5 of the Office Action) the astrocytoma Grade II is a lower grade tumor whereas the Applicants show one instance of this tumor to show a high degree of $\alpha 4$ overexpression. This is exactly the point. Traditional cytology/histology was long used to examine these tumors and establish a ranking. However, it has also been apparent that many malignancies do not behave according to the histologically assigned rank. The apparent discrepancy pointed out by the Examiner is one such instance. The instant invention provides a method of comparing levels of gene expression in a tumor to determine the relative invasiveness or aggressiveness of the tumor. Call it a ranking or grading system, but in any case this determination is intended to replace the traditional histological grading or ranking and is not expected to necessarily agree with that grading or ranking. Applicants have attempted to amend the claim to overcome the rejection under 35 U.S.C. §112, second paragraph; however, suggestions from the Examiner in overcoming this problem are welcomed.

In view of the foregoing, it is respectfully submitted that the application is in condition for allowance. Reexamination and reconsideration of the application, as amended, are requested.

If for any reason the Examiner still finds the application other than in condition for allowance, the Examiner is requested to call the undersigned attorney at the Los Angeles telephone number (310) 734-5200 to discuss the steps necessary for placing the application in condition for allowance.

You are hereby authorized to charge any fees due and refund any surplus fees to our Deposit Account No. 50-2567.

Respectfully submitted,

REED SMITH CROSBY HEAFEY

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By: _____

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Attachment: formal drawings (ten sheets)